Abstract

Cardiovascular diseases are one of the leading causes of mortality among diabetic subjects. Insulin triggers glucose intake through GLUT-4 in metabolic tissues and however, in vascular endothelium insulin mediates vascular homeostasis through the activation of eNOS which subsequently prompts the production of NO. Low grade chronic inflammation is a characteristic feature of type-2-diabetes. Several pro inflammatory cytokines have been shown to induce both metabolic insulin resistance and vascular insulin resistance. Vascular insulin resistance is one of the morbid conditions in T2D, associated with disruption of insulin induced Akt/eNOS signaling axis leading to decreased levels of nitric oxide. Constitutively active pro-inflammatory mediators such as IL-6 has been demonstrated to induce vascular insulin resistance. Further epigenetic mechanisms such as DNA methylation has been shown to contribute to development and progression of Type 2 Diabetes (T2D) and its complications. Thus, in the present study, we aimed to understand the cross talk between insulin, IL-6 signaling pathways and associated DNA methylation changes under the conditions of IL-6 induced vascular insulin resistance. In human endothelial cells (EC), we observed stabilization of IL-6 induced phospho STAT3 Tyr705 in presence of insulin, which might lead to constitutive expression of pro-inflammatory genes. On the other hand, IL-6 impeded insulin response on phospho Akt ser473 and phospho eNOS ser1174 in ECs pretreated with IL-6 indicating vascular insulin resistance due to IL-6. Further, in 3D spheroid and matrigel assays, IL-6 abrogated insulin effects on angiogenesis. HPLC analysis for global DNA methylation resulted in decreased levels of methyl cytosine in cells treated with IL-6. Subsequently, dose dependent and kinetic analysis suggested IL-6 significantly modulated DNMT3B and DNMT1 without affecting DNMT3A protein levels. IL-6 induced reduction in DNMT1 and DNMT3B levels were inversely correlated with S-phase of cell cycle. CpG microarray analysis revealed IL-6 significantly hypermethylated 98 genes and hypomethylated 199 genes. Gene ontology, pathway analysis revealed methylation changes in genes involved in insulin signaling such as IGFR1, MAP3K, RPS6KA2, ITPKB, FOXC2 and FOXD3 were validated by bisulfite DNA sequencing. Taking together, our data indicates causal link between IL-6 induced changes in DNMT1 and DNMT3B leading to demethylation of critical genes and altered gene expression involved in insulin signaling and angiogenesis.